INTERRELATIONSHIPS IN CULTURE AMONG FUNGI ISOLATED FROM SOYBEAN

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RÉSUMÉ. — Sur les 18 espèces isolées des parties aériennes de la plante de Soja et montrant une activité antagoniste, *Penicillium nigricans*, *Cladosporium* sp., *Aspergillus* sp. et *Fusarium* sp. sont les plus actives. L'antagonisme s'observe entre saprophytes et pathogènes, de même qu'entre pathogènes et entre saprophytes. Il peut être dû soit au contact entre les hyphes soit à la compétition nutritive, soit à des métabolites non volatiles. L'âge de la culture, la concentration des métabolites et les conditions de l'environnement influent sur le degré d'antagonisme.

SUMMARY. — Of the several microfungi isolated from the aerial parts of Soybean, only few (18 species) showed antagonistic behaviour and are reported herein. *Penicillium nigricans* and species of *Cladosporium*, *Aspergillus* and *Fusarium* were found to be most successful antagonists. Antagonism was observed between certain saprophytes and pathogens, weak pathogens and non-pathogens and between saprophytes and saprophytes. Besides, intrageneric and intraspecific antagonistic microfungal interactions can be attributed to physical hyphal contact, whereas others are due to nutrients competition or non-volatile metabolites. Parameters used to measure antagonism were zone of inhibition and percentage inhibition of radial growth and/or dry weight of fungi. Age of culture, concentration of metabolites and environmental conditions influenced the degrees of antagonism.

INTRODUCTION

Aerial parts of plants represent distinct ecological niches and support an array of interacting saprophytes and parasites, which determine colonization, onward succession and final equilibrium of microbial populations on these organs (SINGH, 1980). Some microbes create adverse conditions for the others and sometimes for themselves, while there are those which facilitate the growth and multiplication of the neibourouring ones by producing certain stimulating substances (SHARMA et al., 1979, FOKKEMA, 1981). The complexity of such

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interactions on different hosts *in vivo* and under different environmental conditions both *in vivo* and *in vitro* needs to be investigated for their possible use in the biological control of certain epiphytic diseases, an aspect which is still poorly understood (LAST & DEIGHTON, 1965; HEUVEL, 1970, SKIDMORE, 1976; RAI & SINGH, 1980; GUPTA & DIXIT, 1982). With a view to assess the nature of such possible interactions, several microfungi were isolated from aerial organs of *Glycine max* L. and were tested *in vitro*.

MATERIALS AND METHODS

The isolation of micro-fungi were made by dilution plate (SHARMA et al., 1974) and moist chamber (KEYWORHT, 1951) techniques. Pure cultures of fungi used in this study, were maintained on Czapek's Dox Yeast (C.D.Y.) agar medium. The test fungi were grown in dual cultures on agar plates to examine the nature of interactions. Another set of experiment was designed in order to explain the possible mechanism of the interaction observed on agar plate. For this effect of non-volatile metabolites of test fungi on other fungi was screened. Incubation was made in dark at 27 ± 1°C.

Colony interactions were studied using paired culture method (DENNIS & WEBSTER, 1971) in petri dishes (9 cm diam.). Each pair of fungi were inoculated 3 cm apart and five replicate dishes were set up per combination in a petri dish containing about 20 ml C.D.Y. agar. Colony development was recorded daily and assessment made of the interactions when the fungi had achieved equilibrium and there was no alteration in the growth pattern. In general, assessments were made after 7 days. The antagonistic action of a fungus was determined by its ability to develop a zone of inhibition around itself or by forming a boundary line beyond which it will not allow the growth of a competing fungus. Interactions were scored according to the methods and classification of PORTER (1924) and SKIDMORE & DICKINSON (1976).

Parameters used for the measurements of inhibition in dual cultures were the width of zone of inhibition and the percentage inhibition of radial growth $100 \times (r_1 - r_2) / r_1$ where r_1 denotes diam. of the fungus on the unopposed side and r_2 denotes diam. of fungus on the side opposed by the antagonistic fungus (FOKKEMA, 1973).

In order to understand the possible mechanism of the interaction observed on agar plate, effect of 1 %, 5 % and 10 % concentration of non-volatile metabolites of test fungi showing antagonistic action on agar plates, were screened against other fungi on liquid C.D.Y. medium. Instead of changes in radial growth percentage changes in dry weight of treatment experiments over check sets were worked out in Erlenmeyer flasks containing 50 ml liquid C.D.Y. These flasks were inoculated with equal mycelial bits (5 mm) of antagonist and the fungi whose growth was suppressed. Mycelial bits were cut from 7 and 15 days old cultures on C.D.Y. agar media with a sterilized cork borer. Incubation was made at 27 ± 1°C for 12 days. At the end of which mats were harvested

Source : MNHN, Paris

by filtering through whatman filter paper No 42 and were dried at 70°C for dry weight determination. Flasks without metabolites served as controls. Three replicates were made for each treatment.

The inhibition (I) in growth (dry weight) was determined by the following formula : $I = 100 \times \frac{Cg - Tg}{Cg}$

where Cg represents dry weight in check sets and Tg denotes dry weight in treatment sets.

Extraction of metabolites was done firstly in the fungi which showed antagonism in dual agar plates grown on C.D.Y. medium. Secondly, to obtain metabolites of 7 and 15 days old cultures 1 gm each test fungus was centrifuged for 10 minutes at 3000 rpm. After filtration through whatman filter paper No 42, the filtrates were autoclaved at 15 lbs pressure for 10 minutes and were assumed 100 % conc. of non-volatile fungal exudates (metabolites) and antagonist. Dilution with C.D.Y. liquid medium gave graded concentrations of 1 %, 5 % and 10 % metabolites of respective fungus.

RESULTS

Of the several micro-fungi (75 species) isolated from the aerial parts of Soybean only few (18 species) showed antagonism and these were grown in 18 dual cultures on agar plates. Antagonists in question could be classified into five types :

1. Saprophytes antagonistic to weak pathogens; *Penicillium nigricans* Bainier was found to be antagonistic to *Fusarium culmorum* Sacc. and *F. moniliforme* Sheldom whereas *Cladosporium sphaerospermum* Penzig was antagonistic to *Alternaria solani* Sorauer.
2. Weak pathogens antagonistic to non-pathogenic saprophytes : *Fusarium oxysporum* Schlech. and *Rhizoctonia solani* Kochn were observed to be antagonistic to *Aspergillus fumigatus* Fres., *Curvularia lunata* (Wakker) Boedjin, and white sterile mycelium, respectively.
3. Saprophytes antagonistic to saprophytes : several saprophytes showed antagonistic behaviour to other saprophytes. For example, *Penicillium nigricans* Bainier antagonized *Aspergillus flavus* Link., *A. nidulans* (Eidam) Winter, *Curvularia lunata* (Wakker) Boedjin and white sterile mycelium. Whereas, *Cladosporium herbarum* (Pers.) Link was antagonistic to *Alternaria alternata* (Fr.) Keissler and *Epicoccum nigrum* Link. *Cladosporium cladosporioides* (Fresen) de Vries antagonized *Alternaria alternata* (Fr.) Keissler, white sterile mycelium antagonized *Aspergillus nidulans* (Eidam) Winter whereas *Memnoniella echinata* (Rivolta) Galloway and *Stachybrotys atra* Corda were antagonistic to each other.
4. Intrageneric antagonistic antagonists to other species of the same genus : eg. *Aspergillus niger* Van Thiegem and *Aspergillus flavus* Link.

Source : MNHN, Paris

5. Intraspecific antagonist strain antagonistic to other strain of the same species. For instance, *Aspergillus flavus* Link.

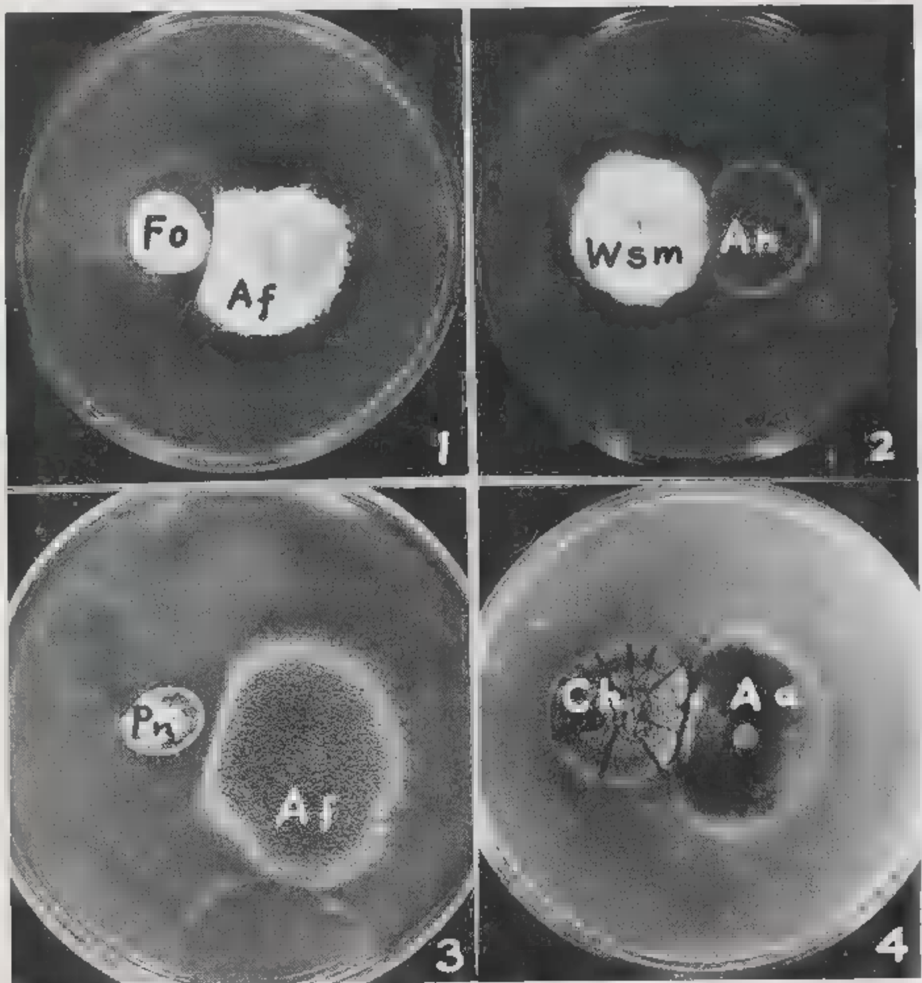


Plate 1 showing antagonism among fungi. — 1 : *Curvularia lunata* (Cl) V/s *Fusarium oxysporum* (Fo). 2 : *Curvularia lunata* (Cl) V/s *Penicillium nigricans* (Pn). 3 : *Fusarium culmorum* (Fc) V/s *Penicillium nigricans* (Pn). 4 : White sterile mycelium (Wsm) V/s *Penicillium nigricans* (Pn).

As it is clear from the Plates 1 & 2, growth was not checked in – *Cladosporium herbarum* V/s *Alternaria alternata* and *Aspergillus nidulans* V/s white sterile mycelium combinations until mycelia of the pathogen and the antagonists came together and there was absence of clearly defined zones of inhibition. In all other cases, growth was prevented at a distance with substantial zones

Source : MNHN, Paris

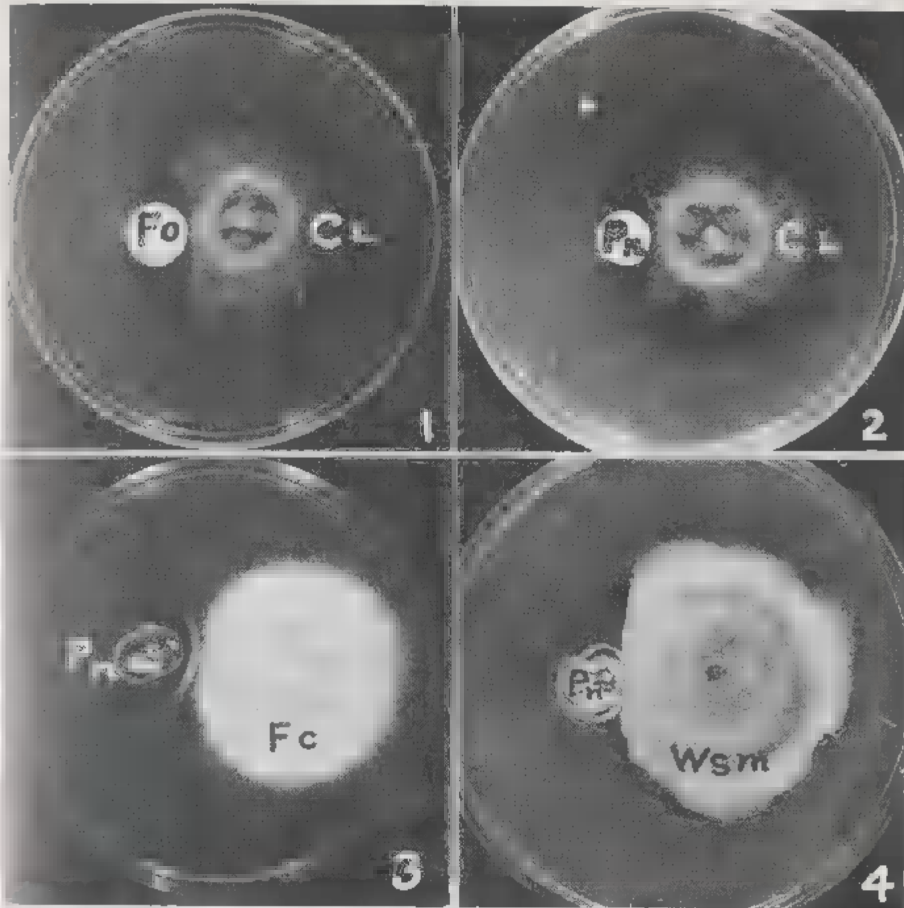
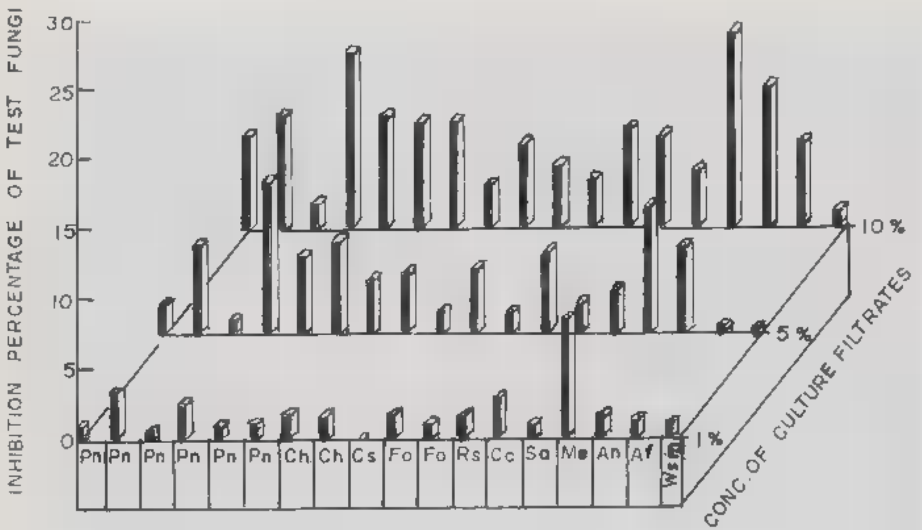


Plate 2 showing antagonism among fungi. — 1 : *Aspergillus fumigatus* (Af) V/s *Fusarium oxysporum* (Fo). 2 : *Aspergillus nidulans* (An) V/s White sterile mycelium (Wsm). 3 : *Aspergillus flavus* (Af) V/s *Penicillium nigricans* (Pn). 4 : *Alternaria alternata* (Aa) V/s *Cladosporium herbarum* (Ch).

of inhibition. Percentage inhibition of radial growth of *Fusarium oxysporum* (Fo), *Curvularia lunata* (Cl), white sterile mycelium (Wsm) and *Aspergillus flavus* (Af) due to the antagonist *Penicillium nigricans* (Pn) was 9.31 %, 26.54%, 17.30% and 10.89%, respectively after 7 days. Antagonistic effects differed according to species of test fungi whereas percentage inhibitions of the radial growths of *Aspergillus nidulans* (An) due to Wsm, of *Alternaria alternata* (Aa) due to *Cladosporium herbarum* (Ch), of *Aspergillus fumigatus* (Af) due to *Fusarium culmorum* (Fc) and of *Curvularia lunata* (Cl) due to *Fusarium oxysporum* (Fo) were 18.80, 19.30, 15.4 and 20.89%, respectively. Interaction

Source : MNHN, Paris



ANTAGONIST FUNGAL EXUDATES

Source : MNHN, Paris

scores of *Penicillium nigricans*, *Fusarium oxysporum* and *Cladosporium cladosporioides* (Cc) and *C. herbarum* were between 4-5 whereas those of other antagonists were between 1-3. Width of zone inhibition (mm) in the antagonistic interactions between *P. nigricans* V/s. *C. lunata*, *F. oxysporum* V/s *A. fumigatus*, *P. nigricans* V/s *F. culmorum* and *F. oxysporum* V/s *C. lunata* (Plate 1 & 2) were 2.5, 1.4, 2.2 and 1.0 mm. respectively.

Data regarding the effect of exudates (non-volatile metabolites) of antagonistic fungi on the percentage of dry weight of those fungi whose growth was suppressed, are presented in Figures 5 and 6. It is clear that, in general, 1) percent reduction in the dry weight of fungi due to antagonist metabolites of 15 days old culture was more substantial than that of 7 days old culture, 2) reduction in dry weight increased gradually with the increasing concentration of culture filtrates of antagonists. However, wise ratio exceptions to above generalization were also observed. *Memnoniella echinata* (Me) and *Cladosporium cladosporioides* were interesting in as much that in the former more growth inhibition was observed in 7 days old culture at 1% conc. whereas in the latter more growth inhibition was observed at 1% and 10% than at 5% conc. (Fig. 5 and 6).

DISCUSSION

During the course of present investigation 18 fungal species were found to exhibit antagonistic action. Out of these, *Penicillium nigricans* and species of *Cladosporium*, *Aspergillus* and *Fusarium* were most successful antagonists followed by *Epicoccum*. Antagonistic actions were observed between certain saprophytic and pathogenic fungi between weak pathogens and non-pathogens and within pathogens. Antagonism was also observed between saprophytes as well as within species and even strains of the same fungus. These observations are in accordance with those earlier reported by TVEIT & WOOD (1955), AHMAD (1970), LAST & WARREN (1972), MUKERJI (1974), SHARMA et al., (1979) and DICKINSON (1981). Additional examples of antagonistic interactions on aerial plant surfaces are documented in DICKINSON & PREECE (1976). The antagonistic role of successful antagonists of the present study, viz., species of *Penicillium*, *Cladosporium*, *Aspergillus* and *Epicoccum* has been recognized by several early workers (HEUVEL, 1970; PACE & CAMPBELL, 1970, SHARMA, et al., 1979). BHATT & VAUGHAN (1963) reported antagonistic actions of *Penicillium* and *Cladosporium* against the pathogens *Botrytis cinerea* and *Alternaria zinniae in vivo* and on agar media. NEWHOOK (1957) found the same action of *Cladosporium* against *B. cinerea*. DIEM (1969) observed the substantial control of *Cochliobolus sativus* whereas SKIDMORE &

Fig. 5 and 6. Percentage inhibition of the growth of test fungi by antagonists 7 and 15 days old culture exudates, respectively.

Source : MNHN, Paris

DICKINSON (1976) and ZWATZ (1976) reported control of *Leptosphaeria nodorum* and *Fusarium* by the antagonist *Cladosporium*. Aspergilli were noted to check the growth of *Helminthosporium spiciferum* by CHAUHAN & GROVER (1973) whereas *Fusarium* was reported as antagonist of *Puccinia penneisetti* by KAPOORIA & SINHA (1969). *Penicillium* as an antagonist has also been reported earlier by SHARMA et al. (1979) and SHARMA & MUKHERJI (1976). RAI & SINGH (1980) reported antagonistic action of *Epicoccum* and *Aspergillus* spp. against the pathogens *Alternaria brassicae* and *Drechslera graminea*.

Some microfungal interactions are due to physical hyphal contact and competition for space and nutrient, whereas others are due to chemical inhibition, toxins or antibiotics. In the present investigation, antagonism between *A. nidulans* and white sterile mycelium, *Cl. herbarum* and *A. alternata*, *Penicillium nigricans* and white sterile mycelium were basically due to hyphal contact. Interactions between *C. lunata* and *F. oxysporum*, *C. lunata* and *P. nigricans*, *F. culmorum* and *P. nigricans*, *F. oxysporum* and *A. fumigatus* and *P. nigricans* and *A. flavus* were basically due to non-volatile metabolites and in the latter, well defined zones of inhibition were demonstrable. Antagonism due to antibiotics, and inhibitory substances (volatile and non-volatile) has already been reported by HEUVEL (1971), DENNIS & WEBSTER (1971). RAI & SINGH (1980) reported that the metabolites of *Aspergillus terreus* and *Cladosporium cladosporioides* are successful inhibitors of the pathogen *Alternaria brassicae* whereas those of *Epicoccum purpurascens* reduce the activity of *Drechslera graminea*. Differences in the antagonistic effects of fungi according to species can be due to the fact that production of metabolites may vary from species to species and between strain to strain of the same species as reported by NORSE (1972). According to DUBES & KESSLER (1963) one or more specific or non-specific metabolic substance(s) may be responsible for antagonism.

Differences in growth reduction due to antagonist in the two methods may be due to altered environmental conditions (pH, temperature and nutrients) as it has been reported by GROVER (1971) and FOKKEMA (1976).

As far as reduction in growth and/or dry weight of fungi due to increasing concentration and age of antagonist is concerned, KAPOORIA & SINHA (1969) and MISHRA & TEWARI (1976) have already reported the increase in inhibition of lesion development of *Puccinia penniseti* and *P. graminis* in proportion to the increasing spore concentrations of the antagonists.

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